N73-13077

NASA TECHNICAL TRANSLATION NASA TT F-14,587

MICROELECTROPHORESIS OF HUMAN ERYTHROCYTES: IMPORTANCE AND USES IN OBSTETRICS AND GYNECOLOGY

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Translation of "La Microelettroforesi dei Globuli Rossi Umani: Importanza ed Applicazioni in Ostetricia e Ginecologia," Ann. Ost. Ginec., Vol. 89, 1967, pp. 118-137.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 DECEMBER 1972

MICROELECTROPHORESIS OF HUMAN ERYTHROCYTES: IMPORTANCE AND USES IN OBSTETRICS AND GYNECOLOGY

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ABSTRACT. On the basis of already acquired ideas about the subject, the authors study the electrophoretic behavior of blood cells during the menstrual cycle, in both normal and pathological pregnancy, and in gynecological affections whether of inflammatory nature or comprising benign or malignant tumors. They describe the method used in detail, and place great importance on same for clinical diagnosis.

Introduction

During a symposium in London in 1963 on the electrophoresis of erythro- /11*
cytes, one of the participating authorities, Ruhenstroth-Bauer from Germany, stated that "cell electrophoresis is in an evolutionary phase, comparable to man's puberty, when it is impossible to determine the exact moment in which he becomes an adult, but it is possible to state that he is gradually moving toward maturity."

This science, in fact, born first as a means of study to determine the surface charge of erythrocytes, today is considerably surpassing the boundaries which it had fixed, and is becoming more important.

Therefore what is, today, the meaning of electrophoresis of erythrocytes?

It represents the only method to establish the type of an isolated cell submitted to a minimum of external influence. Most of the methods used to type a cell employ morphologic criteria for such measurements. However, the determination of the charge intensity of cellular membranes by electrophoresis represents a functional means, although morphologic and functional concepts are tightly bound in the field of molecular biology.

A cell can be typed by determining the density of the elctrophoretic charge, because the great majority of cell types examined to date show values which are characteristic and show very small variations within the same cell type.

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* Numbers in the margin indicate pagination in the foreign text.

Similarly, the configuration of the charge of the cell surface shows a well defined genetic condition.

However, the characteristic charge density is not sufficient to differentiate those types of cells which, for example, have an identical charge density. In this connection it is sufficient to submit these cells before electrophoresis to biochemical and biophysical treatment in order to induce characteristic changes in the electrophoretic mobility. This happens, for example, to hepatic cells in rapid procreation and to ascitic cells of the hepatoma which can be electrophoretically differentiated after incubation with neuraminidase, the enzyme which metabolizes the N-acetylneuraminic acid [1]. All that has /119 been said permits us to hope that in the future, by applying suitable agents to the cell membranes, it will be possible to type all the different cells and produce an anatomic atlas on cell electrophoresis.

This possibility of cell typing opens two further fields of application: one in theoretic biology and the other in practical clinical work.

In fact, these changes which might be induced in cell electrophoretic mobility by biophysical and biochemical means, permit us to deduce other factors on the nature of membrane structure. The same thing is a reality in the clinical diagnostic field of application of this method. When it was discovered that certain diseases are accompanied by characteristic modifications of the electrophoretic mobility of erythrocytes, the electrophoretic study of these cells became a promising diagnostic method.

Historical Notes on the Microelectrophoresis of Red Blood Corpuscles

The movement of cells suspended in a fluid and submitted to the action of an electric field is defined as cell electrophoresis [2, 3].

Though the first observations on this phenomenon go back to the beginning of the last century (Reuss, 1808; Quincke, 1861) and the research done by Helmoltz between 1871 and 1888 had already given a picture of the theory of electrokinetic movement within the actual limits [4], cell electrophoretic phenomena were applied profitably to the differential analysis of various cell groups only by Northrop and Kunitz in 1925 [4] and then perfected by Abramsom, Briggs and Ponder [5; 6, 7] in 1929, 1950 and 1955.

The first study of red blood cells was done in various phases by a certain

number of scholars, such as Abramson, Moyer, Furchgott, Gorin and Ponder [8, 9, 12].

In 1929 [8], it was established that erythrocytes of mammals have a negative charge, that they move in an electric field, and that the mobility varies from type to type, is constant for the same type and not influenced by the dimensions or by the form of the red blood cells.

The mobility of human erythrocytes has been calculated as approximately 1.3 micron per second per volt per centimeter; the mobility of erythrocytes of rabbits, pigs and guinea-pigs is lower; that of the monkey, the hamster and the cat higher; and that of the rat and the mouse similar to man.

Furchgott and Ponder in 1941 established that the surface of erythrocytes is largely composed of lipids with a predominance of phosphorylated acid groups [11].

In another study, Abramson, Gorin and Ponder (1940), came to the conclusion that the surface of erythrocytes is wavy and depressions not deeper than 1 Å [12] can be found.

Various groups of researchers have recently discovered that the electronegative charge, characteristic of red corpuscles, is due in part to carboxylic groups of N-acetylneuraminic acid. This has been demonstrated in electrophoretic research on a series of erythrocytes of different types of animals [13, 16]. Moreover, there is another possible contribution on the part of the alpha-carboxylic group of the glutamic acid [17].

Some interesting data have been collected by studying the changes in mobility caused by antibodies or by viruses absorbed on the surface by erythrocytes [18, 19], and from the changes of the surface induced by X-ray radiations /120 or chemical substances [20, 21]. The isoelectric point of the erythrocytic membrane has been the subject of careful research [10, 22]. However, it is difficult to determine, because at the pH, which is necessary to produce an inversion of the electric charge sign, the erythrocyte is destroyed. Ponder and Ponder, however, believe that they can fix this point at pH 2 or lower [7].

Particles similar in diameter to erythrocytes, suspended in electrophoretic solution and in conditions very close to physiologic ones, have an electrophoretic mobility which depends, above all, on the nature of their lining and not on the form, dimensions or orientability in an electric field [23, 24].

The mobility of erythrocytes suspended in a given buffer is reproducible and characteristic to a given type, but varies from type to type of animals [14, 25]. In the case of human red blood cells the mobility in physiological solution or in phosphate buffer has been demonstrated to be independent from the system of blood collection and from the anticoagulant used [24, 26]. At a high ionic force (> 0.1) erythrocytes show little change in the electrophoretic mobility when relatively high quantities of nonspecific proteins are present [24]. In these conditions it has been demonstrated that mobility is not influenced by albumin, casein, gelatin, fibrinogen and hemoglobin [23].

Electrophoretic Mobility of Erythrocytes in Various Clinic and Experimental Conditions

Fields of application of microelectrophoresis of erythrocytes are many, whether in experimental research on laboratory animals or in clinical research on man.

In the following paragraphs we shall briefly examine all the possibilities of the method's application, and we shall point out the most important results achieved to date by researchers from all over the world.

1. Electrophoretic Mobility of Erythrocytes in Laboratory Animals

As we have already mentioned, the microelectrophoretic mobility of erythrocytes in the rabbit, pig and guinea-pig, is lower than that of erythrocytes in man; on the other hand, the red cells of the monkey, hamster and cat move with a speed higher than human erythrocytes; lastly, erythrocytes of the rat and mouse behave, electrophoretically, like those of man [8]. Therefore, rats and mice are those animals which, with respect to man, furnish the most interesting results.

In experimental pathology very important are the data obtained in viral leukemia of the Friend and Shay type of the mouse: approximately three weeks after injection of the virus and at the same time as the appearance of splenomegaly, an increase of the electrophoretic mobility of erythrocytes can be observed [27, 28, 29]. Analogously, results of great importance have been observed after total radiation in rats with 400-800 r. Erythrocytes of the animals treated in this way increased their speed of movement. This

does not happen if, before radiation, the rats are splenectomized. This behavior is not tied to actinic denaturation of the cell membranes of red corpuscles, because the radiation of rat blood samples with equal doses of r, does not bring about any variation of speed in the blood cells with respect to those not radiated [28].

2. Modifications in the Electrophoretic Behavior of Erythrocytes by Use of Chemical Substances and Enzymes

Electrophoresis permits us to easily obtain information on the composition /121 and structure of the membrane of red corpuscles without having to refer to great alterations or destruction of the cellular organism [24].

Electrophoresis of erythrocytes can be applied after chemical substances or enzymatic reactions have acted upon the cell surface, modifying part of its structure, the chemical composition, or the electric charge [30]. A typical example is given by the treatment of erythrocytes with formaldehyde. This substance causes great electrokinetic stability of the erythrocytes by the formation of crossed protein bonds between the intracellular proteins and those of the membrane, bonds which eliminate the possibility of hemolysis [31].

Erythrocytes treated in this way, do not show any change in the electrophoretic mobility even when modifying the pH and the ionic force of the liquid solution [31]. The addition to a suspension of erythrocytes treated with aldehyde of even a small quantity of hemolysin, restores to each pH and to each value of ionic force the characteristic variations of the electrophoretic mobility of the cells, completely similar to those of untreated erythrocytes [32]. This particular behavior excludes any cationic character of the erythrocytes which, if we consider that they do not undergo modifications in the electrophoretic mobility even after being treated with acetaldehyde, 2-4 dinitrofluorobenzene and p-toluenesulfonylchloride, we can conclude that they behave electrophoretically as a macropolyanion [32].

The valuation of the pK intrinsic to anionic groups of the normal human erythrocyte, at an ionic force of 0.145 grams-ions-liter, is approximately 2.5 and that of human erythrocytes treated with formaldehyde is approximately 2.6 - 2.8 to values of ionic force from 0.0029 to 0.145 grams ions lipo [17].

The presence of these groups of strong acids was observed by Furchgott

and Ponder in 1941, who thought that the charge originated from groups of phosphoric phospholipid acids, more precisely from a cephalin [11].

More recently it has been demonstrated that the electrokinetic modifications of the erythrocytes derive from sialo-mucose acids [17].

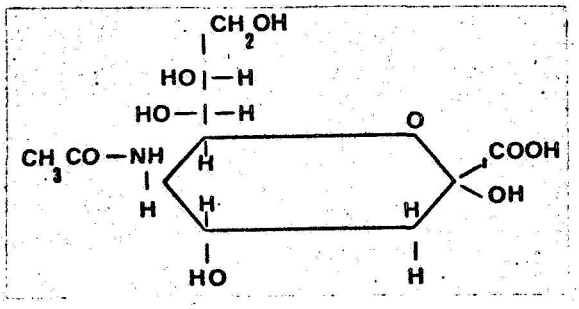
The mucins of the erythrocytic membrane containing sialic acid are known as inhibitors of viral hemagglutination [17].

Sialic acid has been isolated in crystalline form from the erythrocytary stroma by many authors [33, 34].

These researches made Klenk (1958) think that the negative charge of the erythrocytes could be attributed to an acetylneuraminic acid [35]. The presence of N-acetylneuraminic acid on the electrophoretic surface of the red corpuscles has been established above all, by the reduction of the mobility after the removal of the sialic acid resulting from treatment with neuraminidase [17].

The normal erythrocytes and those treated with aldehyde are responsive to the action of neuraminidase, an alpha-glycocide capable of liberating N-acetylneuraminic acid from the proper glycolipin, peptide or protein substrata [17, 35].

The removal of the N-acetylneuraminic acid, which in the case of human erythrocytes has the following structure



brings a reduction of the electrophoretic mobility of erythrocytes equal to 80% of the normal value [15]. The neuraminidase extracted from Vibrio cholerae and from pneumococcus removes approximately 95 to 100% of the N-acetylneuraminic acid of human erythrocytes [14]. From the studies of Eylar et al. (1962) it seems probable that the membrane of human erythrocytes is impermeable to neuraminidase as it is to N-acetylneuraminic acid [14]. /122

The pK of the N-acetylneuraminic acid has been calculated by Svennerholm (1956) to be approximately 2.6 [36]. The proteinases (ficin, papain, tripsin) free mucoids containing N-acetylneuraminic acid from the erythrocytes [37, 38]. The liberation of these sial substances is accompanied by changes of the mobility of erythrocytes [16]. Ponder (1951) for the first had observed the decrease in mobility which happens after the treatment of human erythrocytes with tripsin [39]. Probably this behavior is tied to the proteolysis which brings the removal and/or production of active ionizable groups at the level of the surface of the erythrocyte.

The importance of the above-mentioned factors arises when we consider that the presence of N-acetylneuraminic acid seems indispensable to the appearance of the antigens of the M and N blood groups [40], and that these antigens are destroyed by all the proteolytic enzymes except alpha-chemostripsin [41]. Moreover N-acetylneuraminic acid seems to form the determining group of the Rh_o (D) antigen [42].

Therefore the removal of the sial substances from the ultrastructure of the erythrocyte by the proteolytic enzymes should diminish the affinity of the red corpuscles because of the antibody Rh_o (D) [17].

3. Influence of Antibodies on the Electrophoresis of Red Blood Cells

The electrophoretic mobility of the red cells is reduced after treatment with antibodies. This was demonstrated for the first time by Coulter (1921) and successively tried out by various other researchers (Sachtleben and Ruhenstroth-Bauer, 1962) [22, 43]. Sachtleben (1965) considers that the mechanism of charge reduction by the antibodies consists of the fact that the molecules of the antibodies cover the negative charges of the cell surface. The thickness of the antibody molecule is approximately 40 Å. The covered part of the cell surface and the electric charges joined to it [12] are therefore too much below the new electrokinetic surface to be able to influence the electrophoretic potential [44].

4. Virus Influence on the Electrophoretic Mobility of Human Red Blood Cells

Haning (1948), for the first time, studied the hemagglutination by virus by means of cellular electrophoresis [18]. He found a diminution in the mobility

of human red corpuscles treated with the PR8 influenza virus. The fact that the erythrocytes treated with virus cannot absorb viruses a second time, permits the hypothesis that there are particular structures called "receptors" on the erythrocytic surface capable of catching the virus. As the virus has an enzymatic action, the "receptors" are destroyed by the freeing of neuraminic acid (Klenk, 1955). The neuraminic acid has, under normal conditions, a free carboxylic group. The loss of these charges is indicated as the most probable cause of the diminution of mobility of the red corpuscles [45].

5. Electrophoretic Mobility of Human Erythrocytes

Many authors have tried to establish the value of the normal electrophoretic mobility of human erythrocytes at pH 7. Abramson obtained 1.31 ± 0.02 μ/sec-volt-cm; Furchgott and Ponder 1.02; Crager, Tully and Hansen, using a buffer at pH 7.35, obtained 1.31 ± 0.04 for the A and B groups; 1.52 ± 0.09 for O group and 1.37 ± 0.05 for the AB group. /123

Rottino and Angers obtained 1.32 ± 0.05 in a group of 121 persons and successively 1.27 ± 0.05 in a second group of 76 persons [46].

Further research by many authors established that the electrophoretic mobility of human erythrocytes is considerably lower during pregnancy, in the case of chronic inflammatory processes or in patients with malign neoplasias, while in patients with benign tumors the values are normal [46].

General Principles of Cell Electrophoresis [47]

Most of the isolated cells which are suspended in suitable solutions take an electric charge which can be positive or negative according to the nature of the cells and to certain characteristics of the suspension fluid, but which has always the same sign for all the cells of the same type. Naturally, to this charge, a charge with the opposite sign in the pericellular stratum of the suspension fluid must exist. Introduced into an electric field, each particle, if it is free to move, migrates toward the electrode with the opposite sign (when an electric current is introduced, what counts is the potential difference per centimeter, or, as we say, the field strength and not the intensity of the current).

The charge of the cells refers, as we have said, to an ionogenic quota of

the same, linked to some surface proteins. In itself the electrification of the surface protein molecules has the same origin as any type of salts: only that the particular nature of the radicals, present in great number on their surface (NH, -COOH, -NH, -OH, -SH), and the fact that the electrolytic dissociation of them is influenced differently by the reaction of the means, make it so that to every value of pH corresponds the dissociation of a category and of a well-defined number of ionogenic groups. It follows that the resulting charge can be negative, that is, it determines migration in the anodic direction (in alkaline environment), positive, with cathodic migration (in acid environment), or zero. In this last case the electrophoretic mobility is equal to zero and the value of pH in which this immobility is observed, that is, the point at which the balance of opposite molecular electric forces is established, represents the isoelectric point of the cell. The more we move further away from this value whether in the sense of an increase in concentration of the hydrogen ions or in the sense of a diminution of the same, the greater the charge will become, of which the molecular constituents of the cell are vectors, and consequently the greater will be the migration speed.

The ratio between the electrokinetic properties of a molecule and its charge is rendered more difficult by the fact that the ionized molecule, in order to reestablish its neutrality, encircles itself with a quantity of electro-equivalent ions of the opposite sign furnished by the suspension fluid, ions which arrange themselves around the cell and tend to migrate in the opposite direction, therefore checking their movement.

For this reason the migration speed is never the one that can be calculated, on the basis of Stokes's law, from the charge of the cell, but lower, even when the dimensions of the cell are such that they cannot alter the distribution of the field's lines of force. /124

In addition to the concentration of hydrogen ions (pH), the electrophoretic mobility of a cell depends on the quantity, the strength and the nature of the ions present in the solution.

Therefore the electrophoretic mobility of a cell depends on the pH, the ionic force (established by the concentration and strength of the ions), and

the nature of the ions of the solution; that is, each value of mobility expressed generally in volt/cm, in order to have validity must be accompanied by the indications of these data.

Moreover it is indispensable that the environmental temperature be kept constant as much as possible and that all the readings refer to a standard temperature, normally +25°C. Variations of temperature might bring about changes in the viscosity of the suspension liquid, with alteration to the migratory speed of the cells.

Basically, the apparatus for cellular electrophoresis consists of a transparent chamber with metal electrodes in which we can observe, through a microscope, a suspension of particles in determined conditions. The method was conceived by Ellis (1911-12) [48]. The chamber is normally made of glass and may be cylindrical or rectangular, though a triangular-section type was described by Mitchell (1948) [24, 49].

The electrodes most frequently used for buffers having an ionic force lower than 0.1, are of platinum. For higher ionic forces the electrodes used are of $Zn/ZnSO_4$ or $CU/CuSO_4$ (Abramson, 1929), of $Hg/Hg(NO_3)_2$ (Briggs, 1940), and of Ag/AgCl (James, 1957) [50].

Purpose of the Research

Based on the premises mentioned in the first part of this work, we studied, in order to identify all possible and particular aspects, the behavior of electrophoretic mobility of the red corpuscles of women in various physiological and pathological conditions relating to the branch which is of interest to us.

We have therefore examined the proceeding of electrophoresis of human erythrocytes:

(a) during the menstrual cycle;

(b) during pregnancy, whether normal or complicated by eclampsia, intra-uterine death of the fetus, threat of abortion, Rh isoimmunization, vesicular mole;

(c) tardy pregnancy;

(d) during labor and confinement;

(e) in gynecological inflammatory diseases, benign or malign tumors.

Moreover, in some cases we examined electrophoretic behavior of fetal red corpuscles drawn from the umbilical cord at the moment of birth.

Some of the topics examined by us have been dealt with by other authors.

Abramson [8] found electrophoretic values, considered by him as normal, in four women who had completed the gestation period, and concluded that pregnancy does not influence the electrophoretic properties of red corpuscles.

Rottino and Angers [46], with a total of approximately one hundred women examined, concluded that the electrophoretic mobility of human red corpuscles at pH 7 begins to be lower than normal (1.13 ± 0.03 μ/sec/volt/cm) from the first weeks of pregnancy and remains such until approximately 24 hours after birth. Moreover, the same authors confirm that in gravidal toxemia the electrophoretic mobility of the blood cells increases up to 1.28 ± 0.02 μ/sec/volt/cm.

Rottino and Angers [51] also state that in gynecological malign tumors /125
the electrophoretic velocity of the erythrocytes is considerably lower than normal (0.89 μ/sec/volt/cm) while in cervical inflammations the electrophoretic values fluctuate around 1.14 μ/sec/volt/cm.

Equipment and Methods

1. General

Red corpuscles were collected from 151 individuals (120 women and 31 fetuses). The blood was extracted by a prick in a vein in the arm or directly from the cut funicle and put into polyethylene test tubes containing, as anticoagulant, ethylenediaminotetracetic acid. One cc. of blood of each withdrawal was washed three times with 10 cc. of physiological saline solution. Immediately before beginning the electrophoretic tests, with a pipette for white globules, 2 mm^2 of washed red corpuscles were taken, which were mixed with 25 cc. of Michaelis phosphate buffer at an ionic force of 0.172 and at pH 7.

The technique employed by us has been the one described by Angers and Rottino [52], using the Ponder vertical electrophoretic cell built by the Fisher Scientific Company of New York and allowed to be used by us by the

Societa Polichimica SAP of Milan. To adapt this apparatus to our needs we joined it, in series, with a 25 milliampere amperometer, with a resistance of 4250 ohms, with a 2500 ohms potentiometer and with a polarity converter, to be used in case of necessity. The potential used was 155 volt and the current was 8 milliampere. The microscopic unit (Zeiss company) was formed by a 0.45 mm phase lens (focus = 20); long focus condenser and a 20 enlargement eye-piece with a micrometer screw. The electrodes were made with copper wire (3 mm and a length of 3 cm) situated in the lateral arms of the electrophoretic chamber and filled with saturated solution of $CuSO_4$. The inside of the cell was filled with Michaelis buffer at pH 7, prepared according to the Bull method [53].

2. Procedure

To proceed with the electrophoretic measurements, the red cells, suspended in the buffer, were sucked into a beaker, situated under the electrophoretic chamber. The microscope was focused on the cells (critical point at 0.21 mm from the internal surface of the anterior wall of the cell).

First, the velocity of the fall of the cells, due to gravity, was measured for 5 seconds, then the current was passed for another 5 seconds, always observing the cells' velocity. Subtracting from this velocity the one due to gravity, the velocity of the applied electric force was obtained. To calculate mobility, an average of 5 readings was made which, divided by 5, gave velocity per second. The velocity with which the red corpuscles move per second, per electrical unit, per volume unit of the electrophoretic cell containing the red corpuscles, is called electrophoretic mobility. Calculation of mobility is done in the following way: the mobility of the red corpuscles, suspended in the buffer, is expressed as velocity per potential unit of electric field imposed on the red corpuscles.

The field potential, E, will depend, therefore, on voltage used, on current generated, and on the resistance of the buffer. The correlations are expressed by the formula:

$$\text{Mobility} = \text{velocity}/E = \frac{\text{distance travelled}/\text{time}}{E}$$

The distance is measured by an optical system moving the micrometer screw. /12

The time is 5 seconds and the velocity is calculated on the average of 5 red corpuscles.

Field potential, E, in volt centimeter, is calculated by the formula $E = I\ A\ \lambda$, where I is the current intensity expressed in amperes (in our case = 8×10^{-3} amperes). A is the measure in cm^2 of the area of the section of the electrophoretic cell ($A = 1.1 \times 0.1 = 0.11\ cm^2$), and λ is the specific conductivity of the red corpuscles with a specific buffer. For our Michaelis buffer it was = 1/60. From this we can deduce that if I is constant, E will be

$$E = \frac{I}{A\ \lambda} = \frac{8 \times 10^{-3} \times 60}{11 \times 10^{-2}} = 4.36 \text{ volts/cm.}$$

Typical example:

$$\text{Mobility} = \frac{d/t}{E} = \frac{5.8}{E}\ \mu/\text{sec}$$

$$= \frac{5.8}{4.36} = 1.33\ \mu/\text{sec/volt/cm.}$$

The sensitivity of the method is such that a modification in more or less of pH 7 of a mobility larger than 0.01 μ/sec/volt/cm is of great significance.

3. Results

The results are shown in the following tables I - II - III - IV - V - VI - VII - VIII - IX - X - XI - XII - XIII.

In each table the data related to electrophoretic mobility values of red corpuscles expressed in μ/sec/volt/cm have been preceded by notations relating to the year of observation, the clinical chart, the name, age, patients' parity, and now and again by all those notes useful for the picture of the case.

Table I shows the data relating to the behavior of electrophoretic velocity of red corpuscles in 6 patients during the menstrual cycle. The tests were made on the 1st and 2nd day of menstruation (A), immediately at the end (B), at time of ovulation (C), established by control of the basic temperature, and 5 days after ovulation (D).

Table II examines the results of the tests made on women during the various periods of pregnancy (G), in labor (T), and on the first (P.I) and third (P.III) day of confinement.

Table III considers the relations existing between the electrophoretic mobility of maternal red corpuscles (M) and that of fetal red corpuscles (F).

Table IV reports the data deduced from blood taken from pregnant women beyond the end of gestation, immediately before birth (M) and in their fetuses (F).

Table V reports the electrophoretic mobility values of maternal (M) and fetal red corpuscles (F) in case of eclampsia.

In Table VI we have reported the data relating to electrophoretic mobility of red corpuscles in pregnant women, at various periods of gestation, carrying dead fetuses within the uterus for times varying between 24 hours and 7 days.

Table VII concerns the tests in case of abortion threat.

Table VIII gives the data relating to the electrophoretic mobility of maternal red corpuscles in the case of Rh isoimmunization.

Table IX gives the data relating to two cases of vesicular mole.

In Tables X - XI - XII - XIII the results listed relate to electrophoretic mobility of red corpuscles in patients suffering from inflammatory affections of the genitals (Table X), from uterine fibroma (Table XI), from malign genital neoplasias in various locations (Tables XII and XIII).

TABLE I. ELECTROPHORETIC MOBILITY DURING THE MENSTRUAL CYCLE

(A - Beginning of menstruation; B - End of menstruation; C - Ovulation; D - 5th day after ovulation).

Case	Name	Age	Parity	Electrophoretic mobility			
				A	B	C	D
1	A.M.T.	23	II	1,30	1,30	1,26	1,30
2	F.W.	26	—	1,33	1,33	1,28	1,33
3	N.D.	25	—	1,28	1,28	1,25	1,28
4 (*)	B.M.	43	III	1,34	1,34	1,34	1,34
5	C.G.	26	—	1,30	1,30	1,25	1,30
6	F.A.M.	29	I	1,32	1,32	1,28	1,32
			Average (**)	1,30	1,30	1,26	1,30

(*) In this case the control of the basic temperature did not permit us to put in evidence the ovulation (anovulatory cycle), blood for C and D was taken on the 15th and 20th day of the cycle.

(**) Data of case 4 were excluded from the average.

TABLE II. ELECTROPHORETIC MOBILITY IN NORMAL PREGNANCY (G), IN LABOR (T), ON THE 1st DAY OF CONFINEMENT (P.I), ON THE 3rd DAY OF CONFINEMENT (P.III).

Case	Year	Clinical chart	Name	Age	Parity	Pregnancy month	Electrophoretic mobility			
							G	T	P.I	P.III
1	1965	8250	C.L.	35	I	VII		1,17	1,17	1,30
2	»	8258	N.M.	28	I	VI	1,14			
3	»	8309	R.V.	32	II	VII		1,19	1,19	1,34
4	»	8324	G.A.	33	IV	VI	1.10			
5	»	8576	G.E.	24	—	VIII	1,16			
6	»	8589	B.D.	25	—	VI	1,16			
7	»	9010	E.N.	25	—	IX		1,14	1,14	1,32
8	»	9413	B.M.	30	—	III	1,14			
						IX		1,14	1,14	1,33
9	»	11431	C.M.	29	I	IX		1,13	1,13	1,28
10	»	11440	G.B.	23	I	IX		1,18	1,18	1,32
11	1966	2723	B.E.	26	—	IX		1,16	1,16	1,34
12	»	2802	C.S.	26	III	IX		1,10	1,10	1,33
13	»	2806	M.C.	34	—	VIII		1,14	1,14	1,32
14	»	2828	R.A.	27	I	III	1,12			
						IX		1,12	1,12	1,28
15	»	2941	R.M.	24	—	III	1,18			
16	»	2946	C.T.	31	—	IX		1,16	1,16	1,33
17	»	4006	V.M.	30	I	II	1,17			
						IX		1,17	1,17	1,32
18	»	4512	R.P.	28	—	I	1,14			
						V	1,14			
						Average	1,145	1,15	1,15	1,318

Commas represent decimal points.

TABLE III. ELECTROPHORETIC MOBILITY OF FETAL (F) AND MATERNAL (M) RED CORPUSCLES

Case	Year	Clinical chart	Pregnancy month	Electrophoretic mobility F	M
1	1965	8250	VII	1,34	1,17
2	»	8309	VII	1,34	1,19
3	»	9010	IX	1,30	1,14
4	»	11431	IX	1,36	1,13
5	»	11440	IX	1,38	1,18
6	1966	2723	IX	1,32	1,16
7	»	2802	IX	1,30	1,10
8	»	2806	VIII	1,38	1,14
9	»	2946	IX	1,35	1,16
10	»	4006	IX	1,30	1,17
			Average	1,337	1,154

Commas represent decimal points.

TABLE IV. ELECTROPHORETIC MOBILITY IN LATE PREGNANCY
(MATERNAL RED CORPUSCLES: M)
(FETAL RED CORPUSCLES: F)

Case	Year	Clinical chart	Name	Age	Parity	Electrophoretic mobility M	F
1 (*)	1965	11814	M.A.	26	—	1.23	1,18
2	»	11818	DeP.T.	43	I	1.21	1.09
3	»	11821	B.S.	28	I	1.25	1.10
4 (*)	1966	321	B.V.	27	—	1.21	1,16
5 (*)	»	421	M.G.	27	—	1.25	1.12
6 (*)	»	833	C.L.	36	—	1,21	1,18
7 (*)	»	1249	C.M.R.	34	—	1,17	1.34
8 (*)	»	1557	T.F.	32	—	1,23	1,13
9 (*)	»	1596	G.A.	26	—	1,25	1,18
10 (*)	»	1976	R.A.	28	—	1,25	1,17
11 (*)	»	2407	V.R.	26	—	1,23	1,18
					Average	1,226	1,283

Note: In the designated cases (*) the birth was cesarean.

Commas represent decimal points.

TABLE V. ELECTROPHORETIC MOBILITY IN ECLAMPSIA
(MATERNAL RED CORPUSCLES: M)
(FETAL RED CORPUSCLES: F)

Case	Year	Clinical chart	Name	Age	Parity	Pregnancy month	Electrophoretic mobility M	F
1	1965	8276	M.C.	24	—	VIII	1,21	1,12
2 (*)	»	9544	A.G.	32	I	IX	1,28	—
3	»	10091	R.O.	28	I	IX	1,33	1,04
4	»	10255	P.A.	32	II	VIII	1,23	1,16
5	»	10311	L.R.	21	—	IX	1,19	1,24
6 (*)	1966	78	G.A.	26	—	IX	1,25	1,12
7	»	157	B.A.	26	—	VII	1,28	1,09
8	»	595	S.L.	40	II	VIII	1,14	1,34
9	»	1783	I.G.	26	—	VIII	1,30	1,09
10 (*)	»	1933	I.M.	24	—	VII	1,26	—
11	»	2143	L.V.	26	—	VIII	1,23	1,16
12	»	2203	S.A.	25	—	IX	1,28	1,09
						Average	1,288	1,145

Note: In the designated cases (*) the birth was cesarean.

Commas represent decimal points.

TABLE VI. ELECTROPHORETIC MOBILITY IN INTRA-UTERINE DEATH OF THE FETUS

Case	Year	Clinical chart	Name	Age	Parity	Pregnancy month	Fetus weight	Electrophoretic mobility
1	1965	8234	F.G.	42	XII	VI	1040	1,34
2	»	8745	A.M.	31	—	VIII	2700	1,32
3	»	9244	M.G.	38	—	IX	3600	1,17
4	»	9613	B.F.	43	III	VIII	1900	1,37
5	»	9704	I.F.	27	I	IX	3200	1,32
6	»	9816	B.R.	24	—	VII	2100	1,33
7	1966	3625	R.M.	24	I	VII	1600	1,34
8 (*)	»	3633	C.P.	38	I	VI	920	1,32
9	»	3732	DiB.E.	43	V	V	700	1,28
							Average	1,31

(*) In this case the extraction of maternal blood was done less than 24 hours after the disappearance of the BCF.

Commas represent decimal points.

TABLE VII. ELECTROPHORETIC MOBILITY IN ABORTION THREAT

Case	Year	Clinical chart	Name	Age	Parity	Pregnancy month	Electrophoretic mobility
1	1966	3688	C.A.	37	II	IV	1,14
2	»	3798	DeM.L.	29	I	II	1,16
3	»	3862	L.L.	31	—	III	1,10
4	»	3883	F.A.	38	III	II	1,14
5	»	3885	N.E.	32	I	IV	1,18
6	»	3891	B.A.	37	I	II	1,17
7	»	4200	M.C.	35	I	II	1,14
8 (*)	»	4373	S.C.	30	IV	II	1,19
							Average 1,15

(*) In this case 24 hours after taking the blood the abortion happened.

Commas represent decimal points.

TABLE VIII. ELECTROPHORETIC MOBILITY IN CASE OF Rh ISOIMMUNIZATION

Case	Year	Clinical chart	Name	Age	Parity	Pregnancy month	Anti-body ratio	Electrophoretic mobility
1	1965	8125	M.M.	31	IV	IX	1/128	1,28
2 (*)	»	11673	C.P.	31	II	VIII	1/1024	1,26
3	1966	299	P.A.	29	II	IX	—	1,18
4 (*)	»	1134	S.P.	37	II	IX	1/1024	1,26
5	»	2021	R.M.	24	I	VI	1/256	1,26
6	»	2731	A.L.	25	I	VIII	—	1,18
7	»	3107	S.M.	29	II	IX	1/256	1,26
8	»	3802	P.T.	34	IV	IX	1/128	1,24
9	»	4111	R.L.	30	II	VIII	1,32	1,18
								Average 1,23

Note: In the cases marked by (*) the birth was cesarean.

Commas represent decimal points.

TABLE IX. ELECTROPHORETIC MOBILITY IN CASE OF VESICULAR MOLE
(BEFORE REMOVAL: PS)
(AFTER REMOVAL: DS)

Case	Year	Clinical chart	Name	Age	Parity	Electrophoretic mobility PS	DS
1	1966	1538	L.M.	28	I	0,90	1,09
2	»	3726	Z.ML.	26	—	0,89	—

Commas represent decimal points.

TABLE X. ELECTROPHORETIC MOBILITY IN GYNECOLOGICAL INFLAMMATORY AFFECTIONS

Case	Year	Clinical chart	Name	Age	Diagnosis	Electrophoretic mobility
1	1966	66/Gyn.	C. L.	41	Recurrent adnexitis	1.23
2	1966	116/Gyn.	F. A.	37	Bartholinitis (lat. dextri)	1.27
3	1966	123/Gyn.	F. B.	38	Bilateral adnexitis	1.21
4	1966	148/Gyn.	M. L.	29	Adnexitis (lat. dextri)	1.21
5	1966	179/Gyn.	G. L.	23	Bilateral adnexitis	1.25
6	1966	591/Gyn.	P. V.	28	Bilateral adnexitis	1.23
7	1966	642/Gyn.	F. E.		Pelveoperitonitis	1.17
8	1966	780/Gyn.	C. A.	56	Pelveoperitonitis	1.21
9	1966	781/Gyn.	B. C.	30 28	Inflammatory swelling of the Fallopian tube	1.20
10	1966	1147/Gyn.	S. S.	32	Pelveoperitonitis	1.17
11	1966	1215/Gyn.	S. B.	35	Pelveoperitonitis	1.19
12	1966	1241/Gyn.	C. L.	26	Cervicitis with erosion	1.32
13	1966	1340/Gyn.	B. E.	45	Granuloma of vaginal fornix	1.33
14	1966	4212/Obst.	C. M.	37	Pelveoperitonitis	1.21
15	1966	4405/Obst.	P. I.	40	Endometritis	1.25
16	1966	4429/Obst.	G. B.	27	Peritonitis after recent Cesarean section	1.28
17	1966	4530/Obst.	M. S.	25	Endometritis	1.26
					Average	1.234

TABLE XI. ELECTROPHORETIC MOBILITY IN CASE OF UTERINE FIBROMYOMA BEFORE SURGICAL INTERVENTION (PI) AND AFTER (DI)

Case	Year	Clinical chart	Name	Age	Diagnosis	Electrophoretic mobility PI	DI
1	1966	964	N. G.	51	Uterine fibroma	1.30	1.30
2	1966	1017	A. M.	55	Leiomyoma	1.32	
3	1966	1040	P. E.	53	Multiple fibroids	1.28	1.28
4	1966	1046	Z. M.	51	Multiple fibroids	1.33	1.33
5	1966	1086	D'E. A.	47	Uterine fibroma	1.30	1.30
6	1966	1248	R. V.	46	Uterine fibroma	1.34	
7	1966	1131	C. B.	43	Uterine fibroma; Ovaian cyst	1.33	1.33
8	1966	1133	S. R.	45	Uterine fibroma	1.30	1.30
9	1966	1134	F. A.	44	Large fibroma of the uterine body	1.28	1.28
					Average	1.30	1.30

TABLE XII. ELECTROPHORETIC MOBILITY IN CASE OF GYNECOLOGICAL MALIGN TUMORS (BEFORE ANY SURGICAL INTERVENTION)

Case	Year	Clinical chart	Name	Age	Diagnosis	Electrophoretic mobility
1	1965	2328	V. M.	38	Ovarian K	0.96
2	1965	2329	P. T.	74	Uterine sarcoma	0.92
3	1965	2451	M. A.	49	left ovarian cystoma (arrhenoblastoma)	1.17
4	1965	2678	D. A.	52	K part II B	0.89
5	1965	2689	M. S.	64	Adenocarcinoma	1.00
6	1965	2703	C. L.	47	K part II A	0.89
7	1965	2733	B. P.	75	Adenocarcinoma	1.00
8	1965	2754	S. N.	54	K part I	0.90
9	1965	2769	F. R.	53	Suspected relapsing K part	1.28
10	1965	2784	G. C.	66	Vaginal K	0.89
11	1965	2799	F. G.	55	Adenocarcinoma	0.96
12	1965	2806	M. T.	50	K part II	0.89
13	1965	2808	P. I.	73	Adenocarcinoma	0.89
14	1965	2861	R. M.	52	K part III B	0.89
15	1965	2866	B. A.	38	Adenocarcinoma	0.89
16	1965	2882	B. R.	69	Suspected relapsing K part	0.96
17	1966	1050	R. R.	63	K part II	0.90
18	1966	1055	L. L.	55	K part III B	0.89
19	1966	1087	S. S.	67	K ovarian (right)	0.89
					Average	0.95

TABLE XIII. ANALYSIS OF THE AVERAGE VALUES OF ELECTROPHORETIC MOBILITY IN RED CORPUSCLES IN PATIENTS WITH GENITAL NEOPLASIAS IN VARIOUS LOCATIONS

Case	Diagnosis	Numbers observed	Electrophoretic mobility
1	Cancer of the ovary	3	1
2	Uterine sarcoma	1	0.92
3	Cancer of the endometrium	5	0.95
4	Cancer of the cervix stage I	1	0.90
5	stage II	4	0.89
6	stage III	2	0.89
7	Cancer of the vagina	1	0.89

Results Analysis

In the obstetrics field, examining first the data relating to the tests /133
on electrophoretic mobility of red corpuscles made during the various phases of the menstrual cycle (Table I), we can immediately observe the constancy of the values during the entire cycle, except in the ovulating phase, in which we find an average decrease of 0.04 μ/sec/volt/cm.

In one case only (No. 4), the values remained constant during the entire cycle which, on the basis of the control of the basic temperature, resulted because of anovulation. The average variation of 0.04 μ/sec/volt/cm in the ovulatory phase is completely temporary, but is specific and not to be found in any other physiological condition of woman.

Passing now to consider the data related to pregnancy, normal or pathological, we must give as a premise that the values of electrophoretic velocity of erythrocytes calculated by us in a group of thirty women who were not pregnant nor in the ovulatory phase, and not affected by inflammatory disease or neoplasic, and of an age between 18 and 45 years; results on the average were 1.32 ± 0.02 μ/sec/volt/cm.

During pregnancy, from the initial phases (in a case studied by us in the first month of gestation, electrophoretic velocity was 1.14 μ/sec/volt/cm, Table II, case No. 18) we can observe a considerable decrease of the electrophoretic velocity, which is averaged, as compared to the data of nonpregnant women, lower than 0.17 μ/sec/volt/cm. This decrease remains constant in each case up to the third day of confinement, when the values return to normal levels (1.318 μ/sec/volt/cm).

These data are in complete agreement with what was observed by Angers and Rottino [46].

In late pregnancy, however, the values of electrophoretic velocity of red corpuscles tend to increase before birth; on the average, they are equal 1.226 μ/sec/volt/cm, with an increase, as compared to normal pregnancy values, of 0.081 μ/sec/volt/cm (Table IV).

Red corpuscles of patients affected by eclampsia behave similarly: in this case, though, the increase of mobility is even more evident and on an average,

always in comparison with the normal values of pregnancy, it has increased 0.143 μ/sec/volt/cm (Table V).

In the case of intra-uterine death of the fetus when birth has not occurred, the value of electrophoretic mobility of the red corpuscles has already returned to the nonpregnancy level, if the death of the fetus occurred more than 24 hours before (Table VI).

When there is a threat of abortion, instead, even if it is imminent, we do not find mobility variations with respect to the data relative to normal pregnancy (Table VII).

A considerable increase in mobility (+0.085 μ/sec/volt/cm), always in comparison with normal pregnancy, is also found in the case of Rh isoimmunization. The increase does not seem to have any relation with the antibody ratio (Table VIII). In fact, in a total of 9 women in the pregnancy phase between the 6th and 9th month and with antibody ratios varying between 1/32 and 1/1024, we obtain average values of 1.23 μ/sec/volt/cm. These data are highly significant if we consider that normally the antibodies lower electrophoretic mobility of red corpuscles, as was demonstrated by Coultier in 1921 and successively by Sachtleben and Ruhenstroth-Bauer. Sachtleben (1965) thinks that the charge reduction mechanism on the part of antibodies consists of the fact that the molecules of the antibodies overcome the negative charges of the cell surface. As the thickness of the antibody molecules is approximately /134 40 Å we find that the covered part of the cell surface and the electric charges attached to it are too much below the new electrokinetic surfaces to be able to influence the electrophoretic potential.

Observing instead in our tests that the presence of antibodies in the circulating blood of the patients has increased the electrophoretic mobility of the red corpuscles, it leads us to conclude that the Rh type antibodies, being monovalent or incomplete, behave in a completely different way to the one found by Sachtleben. They, not being agglutinated, do not modify the ionic properties of the cell.

Comparisons which can be made between the values of electrophoretic mobility of maternal red corpuscles and the fetal corpuscles at the moment of birth are of importance. While in normal pregnancy the fetal values are

practically the same as those of women after pregnancy (average 1.337 μ/sec/volt/cm for fetal corpuscles) (Table III), in late pregnancy (Table IV) and in eclampsia (Table V). The fetal red corpuscles show a slowing down with average values of 1.283 μ/sec/volt/cm in late pregnancy, and 1.145 μ/sec/volt/cm in eclampsia.

In the gynecological field, while in benign tumors (uterine fibromyoma) the values of electrophoretic mobility of the red corpuscles are about equal to normal (1.30 μ/sec/volt/cm) (Table XI), in the inflammatory forms of the genitals we find a very characteristic diminution, in comparison to normal, of 0.086 μ/sec/volt/cm (Table X).

Lastly, of great importance are the data in the neoplastic affections of the genitals. Here, in agreement with Rottino and Angers [51], the values of microelectrophoresis are lowered to limits that have not been observed in any other benign affection.

The average value of 0.95 μ/sec/volt/cm with movements from 1 to 0.89 (Tables XII and XIII) has been observed only (Table IX) in two cases of vesicular mole in which, before removal, we had the values 0.90 and 0.89, respectively.

Considerations and Conclusions

During pregnancy the electrophoretic mobility of red corpuscles is approximately 0.2 μ lower than in normal conditions. This figure has been demonstrated to be exact in 100% of the tests made by us and in agreement to what has been observed by other authors (Rottino and Angers [46]).

This change in mobility was observed by us since the end of the first month of pregnancy, but it might occur at an earlier time. We could not always demonstrate it because it was not possible for us to examine patients in an earlier period of pregnancy.

If the pregnancy has a physiological development, the mobility remains low during gestation and during labor, and returns to normal approximately 48 hours after birth.

The presence of living corial villi, due to a particular oxygen tension in the intervillous spaces and the specific modification of the surface of the red corpuscles during pregnancy, imposed above all on the phosphoric acid

groups and the N-acetylneuraminic acid, demonstrated by Eylar et al. [54], could represent the correlated factors of this slowing down. More recently a "slowing factor" has been found in the liquid part of the blood of pregnant women (Sagone - Jurlaro) [56] which must be considered as the most important cofactor in the determination of the slowing down of the electrophoretic mobility of red corpuscles. /135

Also what occurs in late pregnancy, where electrophoretic mobility of maternal red corpuscles, 1.266 μ/sec/volt/cm, can show the importance of the complete vitality of corial villi in maintaining the characteristic mobility of red corpuscles in normal pregnancy. The intra-uterine death of the fetus makes the electrophoretic velocity of maternal red corpuscles return to normal pre-pregnancy values after little more than 24 hours. This fact is certainly exploitable clinically when the intra-uterine death of the fetus is uncertain.

Instead, as a diagnostic criterium for pregnancy, the electrophoretic mobility of the red corpuscles has the disadvantage to be a nonspecific reaction. But it will be interesting to study at which time, after conception, the change of mobility occurs, because this system might be used to identify presumptively the precocious phases of pregnancy.

Also interesting, as collateral diagnostic elements, are the data obtained in Rh isoimmunization in which, though not being able to show a correlation between antibody ratio and electrophoretic mobility, we constantly find, in the presence of antibodies of the Rh type, an increase in the electrophoretic mobility (on the average up to 1.23 μ/sec/volt/cm).

The figure, though found in only two cases, of a considerable lowering of the electrophoretic velocity in cases of vesicular mole, if confirmed by further observation, could be a valid help in the initial cases having a doubtful symptomatology. Lastly, the finding in the gynecological field of normal electrophoretic velocity in benign uterine tumors with values, on the contrary, much lower in the genital cancerous forms (1.30 μ/sec/volt/cm in the former, and 0.95 μ/sec/volt/cm in the latter) and contemporary data which shows in genital inflammatious a certain lowering of the mobility (1.234 μ/sec/volt/cm) not reaching the levels of the cancerous forms, permits us to ascribe to cellular microphoresis a diagnostic possibility of great

importance. In fact, having found electrophoretic values nearly normal (1.28 μ/sec/volt/cm) in a case of suspected relapsing epithelioma of the portio (case No. 9 - Table XII), even before knowing the results of the histological examination, could have permitted us to exclude a relapse.

In conclusion, we can state that more research is needed on the problems tied to microelectrophoresis of red corpuscles, in order to be able to assign to this very fascinating method a reliable diagnostic role, though limited in respect to our discipline, in the fields explored in our study.

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Translated for the National Aeronautics and Space Administration under contract NASw-2038 by Translation Consultants, Ltd., 944 South Wakefield Street, Arlington, Virginia 22204.